REMARKS

Prior to this Response, claims 1-5, 7-9, 12, 14-18, 31, 87, 90, 94-96 and 103 were pending. By the present communication, new claims 104 and 105 have been added. In addition, claims 1, 7-8, 31, 87, 90, 95 and 103 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, upon entry of the amendments, claims 1-5, 7-9, 12, 14-18, 31, 87, 90, 94-96 and 103-105 will be currently pending.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 31 and 95 under 35 U.S.C. § 112, second paragraph as allegedly being indefinite. With regard to claim 31, the Examiner asserts that the term "prior to (b)" confers ambiguity because none of the intervening claims recite a step denoted by the reference "(b)". To overcome the grounds of the rejection, by the present communication claim 31 has been amended to delete the phrase "prior to (b)", thus removing the alleged source of ambiguity from the claim.

With regard to claim 95, the Examiner asserts that omission of proper claim dependency renders the claim "vague". To overcome the rejection, by the present communication claim 95 has been amended to recite dependency from composition claim 87, thus removing the alleged source of vagueness from the claim.

In view of the above described amendments to claims 31 and 95, Applicants submit that claims 31 and 95 meet all requirements under 35 U.S.C. § 112, second paragraph, and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection of Claim 103 under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claim 103 under 35 U.S.C. §112, first paragraph, as allegedly lacking an enabling disclosure. In particular, Applicants traverse the rejection as applied to claim 103 as currently amended.

Applicants respectfully disagree with the Examiner's assertion that the scope of claim 103 is so broad as to encompass "any bone marrow aspirate, any medium comprising any factors and administered [sic] to the heart of any patient" (Office Action, page 6). As currently amended, claim 103 requires that the conditioned medium is obtained by growing autologous bone marrow of the subject to which it is administered. Thus, the donor of the bone marrow is not just "any" subject, but is the subject of the invention method.

Hence, both the source and the subject are defined by the claim language as the same subject. Since this is the required case, Applicants traverse the Examiner's concern in the Office Action that the "*in vitro* examples provided do not address the immune toxicity to any substantial degree." If the bone marrow used is autologous, any factors contained in the conditioned medium are produced by the subject's autologous cells and therefore would not generate an immune response in the subject. Thus, Applicant submits that the Examiner's concerns regarding possible "adverse outcomes related to immunotoxicity" (Office Action, page 6) are unfounded.

In addition, the medium used in the invention method is not "any" medium but is a "growth medium" and can be maintained "under hypoxia" (claim 104). The Specification discloses in Examples 1 and 2, pages 12 and 25, respectively, that appropriate growth medium for growing bone marrow cells is common long term growth medium under normoxic conditions (5% CO_2 at 33° C in T-75 culture flasks) or "hypoxia" (growth in a chamber containing only 1% oxygen) (Example 2). Alternatively, the growth medium can comprise an effective amount of at least one angiogenesis stimulating cytokine selected from GM-CSF,

MCP-1, EPAS1 and HIF-1. Thus, the growth medium as required in presently amended claim 103 is clearly defined.

The Examiner further asserts that there are no animal models disclosed wherein the culture medium is "undefined" or where the source of the bone marrow aspirate can be from "any" organism. The source of the bone marrow aspirate and definition of the growth medium are addressed above. With regard to animal studies, Applicants have elsewhere demonstrated by ELISA that cells in bone marrow aspirate when grown in culture produce conditioned medium comprising a complex variety of endogenously expressed cytokines at defined concentrations in a dose dependent manner. (See Exhibit A attached, T.D. Kinnaird et al. "Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms" *Circulation*, 2004;109:1543-1549, especially table, page 680). This article also shows that *conditioned medium alone* "exerts in vitro biological effects relevant to collateral remodeling" and describes injection of *conditioned medium alone* into ischemic adductor muscle of mice that resulted in "significant improvement of [blood] flow by day 3, which [improvement] was maintained for the duration of the study" (Kinnaird, page 681, col. 2, bottom). Thus, direct administration of conditioned culture medium (which inherently contains endogenous agents produced by growth and expansion of unfractionated bone marrow cells in vitro) results in the claimed result of enhanced collateral vessel development in hind limb in a murine animal model.

The bone marrow used to prepare the conditioned medium is "autologous bone marrow aspirate," i.e., containing an unfractionated cell population. As is explained in more detail below, Applicants submit that the term "aspirate" as used in claim 103 (and throughout the claims) means a composition comprising an unfractionated bone marrow cell population. The Specification discloses filtration of the "aspirate" to remove bone chips and agglomerates or to obtain the cells therefrom (Specification, page 12, first paragraph) depending upon the size of the filter used, but the Specification does not teach preparation of a particular cell population from the bone marrow for culture. Since the bone marrow aspirate is unfractionated with

respect to cell populations, the Examiner's concerns regarding possible unpredictability introduced by claim 103 failing to specify a particular population or subpopulation of cells grown in the growth medium are unfounded. All the bone marrow cells present in the aspirate are grown together and allowed to express endogenous product into the growth medium in a time appropriate manner that is determined by the cells themselves. Product of the marrow-derived stromal cells in the growth medium would therefore be present as in the experiment of Kinnaird et al. where enhanced collateral vessel development was shown in mouse hind limb.

The Examiner also states as further grounds of the rejection for alleged lack of enablement: "[T]here no animal model is presented wherein conditioned culture medium alone is administered to the animal's heart" (Office Action, page 6). With reference to this concern of the Examiner, Applicants point out that Mickle et al. (of record as cited by the Examiner herein) discloses injection into heart of conditioned medium formed by culture of mesenchymal stem cells (as a control) (Mickle et al., ¶ [0043]). More recently, Gnecchi et al., "Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells" (2005) *Nature Medicine* 11(4):367-8), attached as Exhibit B) have shown in a in vivo murine heart model that injection of Akt-concentrated conditioned medium obtained from growth of bone marrow cells, especially hypoxic bone marrow cells, exerted a protective effect upon ischemic myocardium which the authors attributed to paracrine signaling by factors released into the conditioned medium by the cells. Gnecchi et al. looked in particular at the protective effect of paracrine signaling in the context of its preservation and repair of numbers of cardiomyocytes, rather than its effect upon collateral development of vessels in ischemic tissue. However, despite the differences between the Gnecchi et al. and Mickle et al. methods and the invention method as described in the Specification and defined by amended claim 103, Applicants submit that these references corroborate the safety of direct injuction of conditioned medium into heart tissue and answer the Examiner's concern that none of the animal models of record disclose administering conditioned culture medium alone to the animal's heart since both Mickle et al. and Gnecchi et al. describe a therapeutic an animal model wherein

conditioned medium of bone marrow aspirate derived cells alone is administered to the animal's heart.

In view of the amendments to the claims discussed above and the above remarks, Applicants believe that the concerns of the Examiner regarding the scope of the claims and the need for animal models to illustrate the methods of claim 103-105 have been met sufficiently that enablement of claims 103-5 is established under 35 U.S.C. § 112, first paragraph. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection for alleged lack of enablement.

The Rejections under 35 U.S.C. § 102 and 102(e)

A. Applicants respectfully traverse the rejection of claims 87, 94 and 95 under 35 U.S.C. § 102(b) as allegedly being anticipated by Nakahata (U.S. Patent No. 5,610,056, hereinafter "Nakahata"). As currently amended, the invention composition distinguishes over Nakahata by requiring: "A composition for enhancing collateral blood vessel development comprising cultured autologous bone marrow aspirate that has been stimulated ex vivo by exposure to hypoxia or further comprises an effective amount of at least one angiogenesis stimulating cytokine selected from GM-CSF, MCP-1, EPAS1 and HIF-1.

Applicant respectfully disagrees with the Examiner's assertion that the term "bone marrow aspirate" is not particularly limiting, thus is interpreted as broadly as reasonable to mean any population of bone marrow cells" (Office Action, page 7). Applicant has used the term "autologous bone marrow aspirate" to indicate "fresh aspirate" or autologous bone marrow that is unfractionated with respect to cell contents. The Specification describes "freshly aspirated" bone marrow being filtered to remove bone particles and agglomerations or to separate cells from other content in the aspirate, but nowhere does the Specification describe the cells being separated into any particular cell type, such as hematopoietic stem cells or progenitor cells. Thus, the term "bone marrow aspirate" as used in the composition claims encompasses the case

wherein all types of cells in the bone marrow are present. This meaning for the term "aspirate" is consistent with the common dictionary meaning of the term, e.g., that which is withdrawn by an aspirator. Use of bone marrow aspirate rather than a particular sub population thereof considerably streamlines the process for preparing the invention composition and is considered by Applicants to be a significant novel feature of this invention.

Thus, Applicant submits that the Examiner's interpretation of the term at issue to mean "any population of bone marrow cells is not "reasonable" in view of the common meaning of the term "aspirate."

By contrast, Nakahata fails to disclose a composition that comprising cultured autologous bone marrow aspirate that has been stimulated ex vivo by exposure to hypoxia or further comprises an effective amount of at least one angiogenesis stimulating cytokine selected from GM-CSF, MCP-1, EPAS1 and HIF-1. Instead Nakahata discloses: "Normal human hematopoietic stem cells were isolated from cord blood mononuclear cells and were cultured in the presence of varying concentrations of sIL6R, together with IL-6 and SCF." (Nakahata, col. 2, lines 55-59). In addition, the culture medium employed by Nakahata was "serum-containing culture" (col. 3, line 19) or in "serum-free culture", but Nakahata is absolutely silent regarding use of growth culture under hypoxia or with addition of at least one cytokine selected from GM-CSF, MCP-1, EPAS1 and HIF-1. Thus, Nakahata fails to disclose significant elements of the invention composition, i.e., autologous bone marrow aspirate and the particular cytokines recited by Applicants as being present in the culture medium. Similarly, Nakahata fails to disclose the composition of claims 94 and 95, which contain all limitations of claim 87.

Accordingly, Applicants respectfully submit that each and every element of the invention composition, as defined by amended claim 87 and claims dependent thereon, is not disclosed by Nakahata, as is required to show anticipation under 35 U.S.C. §102. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

B. Applicants respectfully traverse the rejection of claims 87, 94 and 95 under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 5,997,860 to Bauer et al. (hereinafter "Bauer et al."). As currently amended, the invention composition distinguishes over Bauer et al. by requiring: "A composition for enhancing collateral blood vessel development comprising cultured autologous bone marrow aspirate that has been stimulated ex vivo by exposure to hypoxia or further comprises an effective amount of at least one angiogenesis stimulating cytokine selected from GM-CSF, MCP-1, EPAS1 and HIF-1.

Applicants disagreement with the Examiner's interpretation of the scope of the invention composition claims, which is set forth above in Section A, applies equally and is incorporated here. Applicants submit that Bauer et al. fail to disclose at least one element of the invention composition, as recited by amended claim 87. The Examiner asserts that Bauer et al. disclose a culture comprising autologous bone marrow where cells are exposed to cytokines so as to stimulate the cells. However, Bauer et al. fail to disclose a composition that comprises cultured autologous bone marrow aspirate that has been stimulated ex vivo by exposure to hypoxia or further comprises an effective amount of at least one angiogenesis stimulating cytokine selected from GM-CSF, MCP-1, EPAS1 and HIF-1. Instead Bauer et al. disclose a composition wherein at least one variant of hIL-3, which may be combined with a number of different cytokines, is always necessary in the composition. Nowhere do Bauer et al. disclose a composition with properties for enhancing development of collateral blood supply in heart or limb tissue or one wherein the composition is not required to contain at least one variant of hIL-3. In addition, Bauer et al. are completely silent regarding stimulation of autologous bone marrow aspirate by exposure ex vivo to hypoxia. Thus, Bauer et al. fail to disclose each and every element of Applicants' claim 78, as would be required to show anticipation under 35 U.S.C. °§ 102(e). Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

C. Applicants respectfully traverse the rejection of claims 1-4, 14-16, 18, 87, 94-96 and 103 under 35 U.S.C. § 102(e) as allegedly being anticipated by Mickle et al. (U.S 2005/0032600 A1; hereinafter "Mickle et al.")

As currently amended, the invention method of enhancing collateral blood vessel development in a subject, as defined by amended claim 1, distinguishes over Mickle et al. by requiring administration to sites in heart or limb tissue an effective amount of autologous bone marrow aspirate (i.e., in the absence of at least one of stimulatory cytokines such as are required in claims 7-9).

The invention composition for enhancing collateral blood vessel development, as defined by amended claim 87, distinguishes over Mickle et al. by requiring "cultured autologous bone marrow aspirate that has been stimulated ex vivo by exposure to hypoxia or [which] further comprises an effective amount of at least one angiogenesis stimulating cytokine selected from GM-CSF, MCP-1, EPAS1 and HIF-1". Applicants note that claim 87 omits any requirement that the composition contain a pharmacological drug, such as 5-azacytidine.

The invention method of enhancing collateral blood vessel formation in a subject in need thereof, as defined by amended claim 103, distinguishes over the disclosure of Mickle et al., by requiring "directly administering to ischemic sites in heart or limb tissue of the subject an effective amount of conditioned medium in which autologous bone marrow aspirate has been grown in cell growth medium under hypoxia or wherein the growth medium comprises an effective amount of at least one angiogenesis stimulating cytokine selected from GM-CSF, MCP-1, EPAS1 and HIF-1 to induce collateral blood vessel formation in the heart or limb tissue."

Applicants again disagree with the Examiner's interpretation of the term "aspirate" as meaning "any bone marrow aspirate, from which any bone marrow cell population is derived" (Office Action, page 10). Applicants respectfully submit that the Examiner's statement is self-contradictory. A bone marrow cell population derived from bone marrow aspirate is necessarily narrower in scope than is "bone marrow aspirate", which includes all cell populations aspirated

from the bone marrow, but with removal of fat and debris, such as bone fragments. Thus, bone marrow aspirate encompasses, i.e., "reads on", any sub-population of bone marrow cells, but is of much broader scope than any subpopulation of cells. Applicants submit that the narrower term (the species) does not anticipate the broader term (the genus).

In addition, while the Specification describes how to obtain the cell contents of bone marrow aspirate (see cite above), the Specification emphasizes the importance of "taking advantage of the natural ability of [bone marrow] cells to secrete many angiogenic factors in a time-appropriate manner [to] provide an optimal intervention for achieving therapeutic collateral development in ischemic myocardium" (Summary, page 3, final paragraph). See also Applicant's teaching that use of the cells per se could prove a more sustained sour of these natural angiogenesis agents (Specification, page 7, lines 10-11). In fact, the invention is based on the premise that cells in bone marrow aspirate as a group secrete a plurality of "combined" agents (page 4, lines 5-9) and hence can be exploited in therapeutic applications with improved result over prior art techniques that employ a single angiogenic cytokine. This is the crux of the invention. Thus, Applicants respectfully submit that it flies in the face of the basic concept of the invention to interpret "aspirate" as meaning an individual cell population extracted therefrom.

With this concept in mind, it is easy to see that Mickle et al. fail to disclose each and every element of claims 1, 87 and 103 because Mickle et al. are absolutely silent regarding administration to a subject of bone marrow aspirate that has not been exposed to added stimulatory cytokine, any composition comprising bone marrow aspirate, e.g., the full spectrum of bone marrow cells, or administration to a subject of conditioned medium produced by culture of bone marrow aspirate. In addition, the reference is silent regarding the stimulatory effect on bone marrow aspirate (or any cell fraction thereof) of exposure in culture to hypoxia or one or more of GM-CSF, MCP-1, EPAS1 and HIF-1.

Accordingly, Applicants submit that Mickle et al. fail to disclose each and every element of amended claims 1, 87 and 103 (and claims dependent thereon), as is required to establish

anticipation under 35 U.S.C. § 102(e) and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 103(a)

Applicants respectfully traverse the rejection of claims 1-5, 14-16, 18, 87, 94-96 and 103 under 35 U.S.C. § 103(a) as allegedly being anticipated by Mickle et al. and further in view of Mack et al. (J. Thorac. Cardiovas. Surg. (1998) 115:168-77; hereinafter "Mack et al."). The discussion above of the deficiencies of Mickle et al. for anticipating the elements of claims 1, 87 and 103 applies equally and is incorporated here in its entirety. In addition, Applicants submit that the presently pending claims distinguish over the combined disclosures of Mickle et al. and Mack et al. under 35 U.S.C. § 103(a).

Applicants submit that Mickle et al. would fail to suggest to those of skill in the art the invention method of enhancing collateral blood vessel development in a subject by administration to heart or limb tissue of the subject of "fresh" bone marrow aspirate, i.e., containing the full set of bone marrow cell populations, to which no stimulatory cytokines have been added, the composition of claim 87 comprising bone marrow aspirate and at least one of GM-CSF, MCP-1, EPAS1 and HIF-1; or the method of enhancing collateral blood vessel formation in a subject in need thereof, as defined by amended claim 103, wherein ischemic sites in heart or limb tissue of the subject are administered an effective amount of the recited conditioned medium to induce collateral blood vessel formation in the heart or limb tissue. Mickle et al. are primarily concerned with stimulating cardiomyogenesis in damaged heart tissue. For this purpose, Mickle et al. disclose the stimulatory effect of 5-azacytidine, or an analog thereof, on mesenchymal stem cells, which are not necessarily derived from bone marrow, to induce differentiation of the cells into cardiomyocyte-like cells to restore muscle function to scar tissue. Injection into damaged heart tissue of conditioned medium presumably obtained by culture of MSC's and 5-azacytidine is disclosed by Mickle et al. only as a control (Mickle et al.,

¶ [0043]). Mickle et al. are silent regarding a method of administering "fresh" bone marrow aspirate for enhanced perfusion (whether due to increase in cardiomyocytes or development of collateral flow) in heart or limb tissue or a composition containing fresh BM aspirate that has been exposed to hypoxia or the claimed stimulatory cytokines to stimulate cells in bone marrow aspirate. Disclosure pertaining to "angiogenesis" is summarized in Fig. 5 and the analysis thereof in the cited reference, wherein injection of "fresh MSC's" or "cultured MSC's" are disclosed to achieve a higher density of capillaries in treated tissue than is obtained by injection of MSC's stimulated with 5-azacytidine. In view of the data summarized in Fig. 5, Applicants submit that nothing in the reference itself suggests modifying the disclosure therein along the lines of Applicants' invention, e.g., stimulating bone marrow aspirate (rather than a particular cell fraction thereof) by exposure to hypoxia or GM-CSF, MCP-1, EPAS1 or HIF-1 to enhance "angiogenesis" in heart or limb tissue. In fact, if anything, the data presented by Mickle et al. actually lead away from the invention by showing that cytokine stimulation of MSC's decreases angiogenesis in heart scar tissue.

The Examiner relies upon the combined disclosures of Mack et al. and Mickle et al. for rendering obvious the present invention. In particular, the Examiner points out that Mack et al. disclose a catheter-based delivery system to deliver vector-encoded VEGF to promote angiogenesis in ischemic heart tissue and asserts: " [W]here an angiogenic factor(s) is being administered to a subject's heart, Mack et al. teach that to obviate non-targeted angiogenesis direct delivery is preferred" (Office Action, page 13). However, Applicants submit that there is no motivation provided by the combination of Mack et al. and Mickle et al to substitute hypoxia or any of GM-CSF, MCP-1, EPAS1 or HIF-1 for VEGF to promote angiogenesis and there is no motivation provided by the combination of the cited art to substitute in-culture cytokine-stimulation of bone marrow aspirate for cytokine-stimulation of MSC's as taught by Mickle et al. In fact, in view of Mickle et al.'s showing that cytokine stimulation of MSC's is effective for promoting cardiomyogenesis but provides no advantage for angiogenesis in heart tissue, Applicants respectfully submit that even if those of skill in the art were motivated to combine the

disclosures of the cited art, the present invention, as defined by claims amended claims 1, 87 and 103, would not be suggested.

Accordingly, Applicants respectfully submit that the combined disclosures of Mickle et al. and Mack et al. do not establish *prima facie* obviousness of claims 87 and 103 under 35 U.S.C. § 103(a) and reconsideration and withdrawal of the rejection are respectfully requested.

Allowable Subject Matter

The Office Action indicates that claims 7-9, 12, 17 and 90 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including the limitations of the base claim and any intervening claims. However, in view of the amendments and the arguments above, Applicants submit that all claims are now in condition for allowance. Therefore, Applicants respectfully request withdrawal of the objection to claims 7-9, 12, 17 and 90.

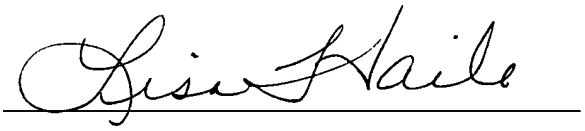
Conclusion

In view of the above amendments and remarks, Applicants submit that all objections and rejections are now overcome, and passage of the claims to allowance is respectfully requested.

If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: June 28, 2005



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Attachments:

Exhibit A Kinnaird et al, "Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms" (2004) *Circ Res.* Mar 19; 94(5):678-85.

Exhibit B Gnecchi et al., "Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells" (2005) *Nature Medicine* 11(4):367-8.